Bacterial Efflux Pump Inhibitors from Edible Plant Sources and Aptamers

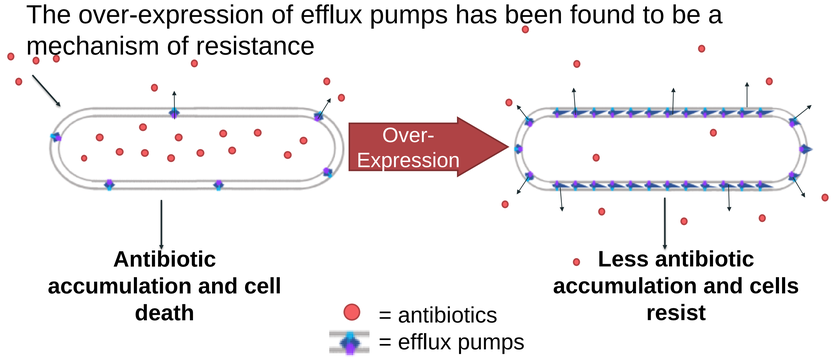
Sarah Baxter
Advisor: Dr. Matt Lopper
University of Dayton Chemistry Department

Abstract

Multidrug resistant bacteria have become a great concern in the world of medicine. Antibiotics are not being discovered at a fast enough rate to fight this resistance, leaving many bacterial infections left unable to be treated with the current antibiotics. The efflux of drugs out of cells is one of the mechanisms contributing to this resistance. Transporter proteins called efflux pumps, located in the membranes of bacterial cells, are responsible for this antibiotic exporting activity. In drug resistant bacterial cells, efflux pumps can expel antibiotics out of the cell, making it difficult for the bacteria to receive the intended dose of the drugs. I have investigated the bacterial efflux pumps and their role in conferring multidrug resistance. I sought to identify new efflux pump inhibitors through the screening of extracts made from edible plants and through the development of synthetic nucleic acid aptamers that bind to the efflux pumps and block their activity.

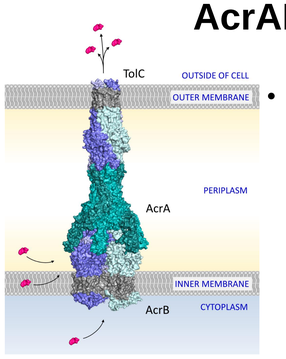
Antibiotic Resistance

- Over 2 million people are infected with resistant bacteria and at least 23,000 die as a result of these infections each year (CDC)
- 700,000 deaths globally from AMR
- AMR: microorganisms unable to be killed with antibiotics, antivirals, antifungals, and other drugs due to resistance mechanisms
- The over-expression of efflux pumps has been found to be a mechanism of resistance



Hypothesis: Can we identify efflux pump inhibitors from edible plant sources and synthetic nucleic acid aptamers?

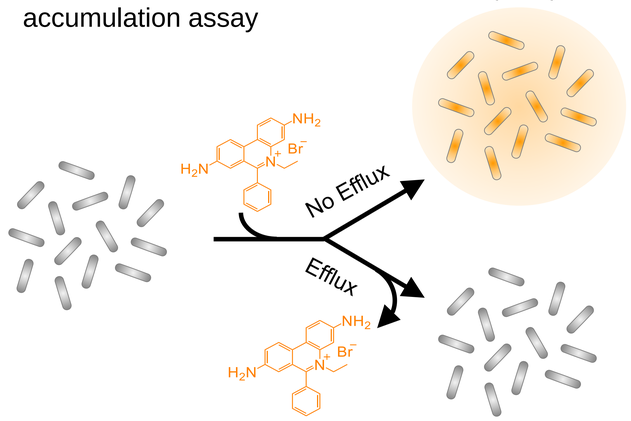
AcrAB-TolC Efflux Pump



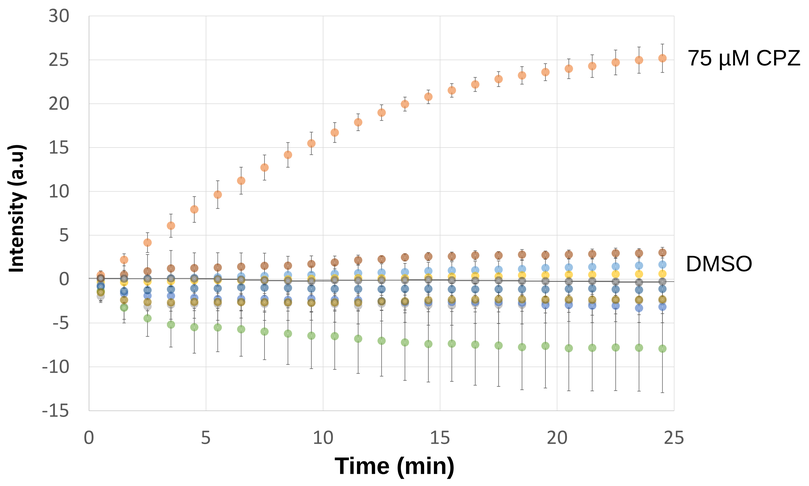
- 3-component pump found on membrane of a bacterial cell
  - AcrA-adaptor proteins
  - AcrB-pump powered by proton motive force
  - TolC-hollow tube-like channel that penetrates the surface of the bacterial cell

Inhibitors from Edible Plant Sources

- Extracted organic compounds from 6 different edible plant sources (cauliflower, portabella mushrooms, asparagus, lavender, hemp seeds, and brown flaxseeds)
- Tested extracts in an ethidium bromide (EtBr) accumulation assay

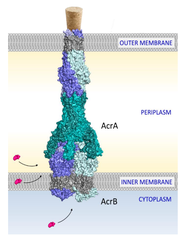


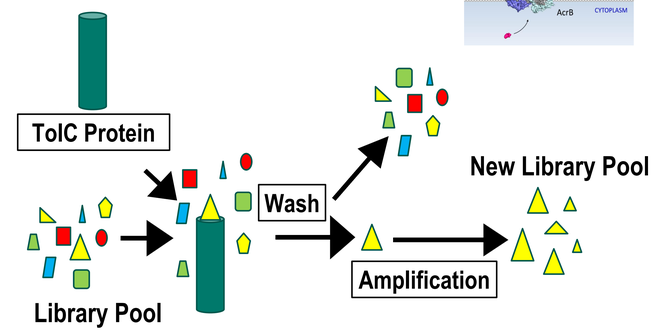
Plant Source Results



Inhibitors from Aptamers

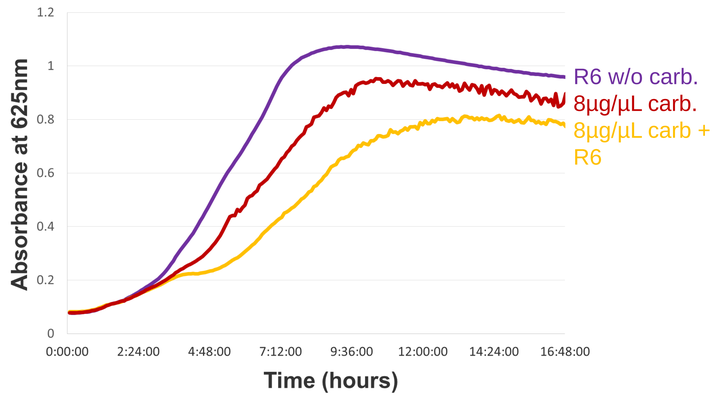
- Goal: to design ssDNA fragments, using SELEX, to bind to TolC and block efflux pump activity





- Repeated a total of 10 rounds of SELEX and tested Rounds 6-10 in a broth microdilution using carbenicillin

Aptamers sensitize *E. coli* to carbenicillin



Conclusions

- Plant extracts closely resembled the DMSO, solvent control. Efflux activity was not blocked by any extracts
- Round 6 Aptamers made carbenicillin more effective against *E. coli*

References

- Centers for Disease Control and Prevention. (2013). Antibiotic / Antimicrobial Resistance. Retrieved August 20, 2017, from https://www.cdc.gov/drugresistance/index.html
- Coldham, N. G., Webber, M., Woodward, M. J., & Piddock, L. J. V. (2010). A 96-well plate fluorescence assay for assessment of cellular permeability and active efflux in Salmonella enterica serovar Typhimurium and Escherichia coli, (May), 1655–1663. https://doi.org/10.1093/jac/dkq169
- Prasch, S., & Bucar, F. (2015). Plant derived inhibitors of bacterial efflux pumps : an update. *Phytochemistry Reviews*, *14*(6), 961–974. https://doi.org/10.1007/s11101-015-9436-y
- Symmons, M. F., Bokma, E., Koronakis, E., Hughes, C., & Koronakis, V. (2009). The assembled structure of a complete tripartite bacterial multidrug efflux pump, *2009*, 26–31.